Antimicrobial Wine Formulations Active Against the Foodborne Pathogens *Escherichia coli* O157:H7 and *Salmonella enterica*

MENDEL FRIEDMAN, PHILIP R. HENIKA, CAROL E. LEVIN, AND ROBERT E. MANDRELL

ABSTRACT: We developed wine formulations containing plant essential oils and oil compounds effective against foodborne pathogenic bacteria *Escherichia coli* O157:H7 and *Salmonella enterica*. HPLC was used to determine maximum solubility of antimicrobials in wines as well as amounts of antimicrobials extracted by wines from commercial oregano and thyme leaves. Activity of essential oils (cinnamon, lemongrass, oregano, and thyme) and oil compounds (carvacrol, cinnamaldehyde, citral, and thymol) in wines were evaluated in terms of the percentage of the sample that resulted in a 50% decrease in the number of bacteria (BA_{50}). The ranges of activities in wines (30 min BA_{50} values) against *S. enterica/E. coli* were carvacrol, 0.0059 to 0.010/0.011 to 0.021; oregano oils, 0.0079 to 0.014/0.022 to 0.031; cinnamaldehyde, 0.030 to 0.051/0.098 to 0.13; citral, 0.033 to 0.038/0.060 to 0.070; lemongrass oil, 0.053 to 0.066/0.059 to 0.071; cinnamon oil 0.038 to 0.057/0.066 to 0.098; thymol, 0.0086 to 0.010/0.016 to 0.022; and thyme oil, 0.0097 to 0.011/0.033 to 0.039. Detailed studies with carvacrol, the main component of oregano oil, showed that antibacterial activity was greater against *S. enterica* than against *E. coli* and that wine formulations exhibited high activities at low concentrations of added antimicrobials. The results suggest that wines containing essential oils/oil compounds, added or extracted from oregano or thyme leaves, could be used to reduce pathogens in food and other environments.

Keywords: antibacterial activities, *Escherichia coli* O157:H7, essential oils, HPLC, microbial food safety, oregano and thyme leaves, *Salmonella enterica*, wines

Introduction

Humans have consumed wine produced by fermentation of grapes for about 7000 y (Ben-Noun 2002; Adams and others 2004). The acidity, organic acid content, alcohol content, and the content of polyphenolic and flavonoid compounds including tannins and resveratrol may be responsible for the reported antimicrobial activities of wines against foodborne pathogens.

Reported studies on antimicrobial effects of wines include the following observations: (1) wine was effective in vitro in reducing the number of viable *Chlamydia pneumoniae, Escherichia coli, Helicobacter pylori, Listeria monocytogenes, Salmonella enteritidis, Salmonella typhimurium, Shigella dysenteriae*, and *Yersenia enterocolitica* as well as reducing virulence of the toxin secreted by the *H. pylori* (Weisse and others 1995; Corona and others 2001; Daroch and others 2001; Just and Daeschel 2003; Mahady and others 2003; Schriever and others 2003; Tombola and others 2003); (2) both red and white wines killed *Salmonella* in vitro but were not effective in preventing infection in mice (Sugita-Konishi and others 2001); (3) wines had little effect against *E. coli* in a model stomach system containing food and synthetic gastric fluid, but killed all the *S. typhymurium* bacteria (Just and Daeschel 2003); and (4) the antibacterial activity of wines may be largely due to synergistic effects of the acidic pH, organic acids (malic, tartaric), and alcohol of wines (Moretro and Daeschel 2004).

The question arises as to whether wine formulations containing nontoxic, food-compatible, plant-derived antimicrobials can be devised to improve microbial food safety. In an effort to define the chemical basis for the antimicrobial activities of natural products, we previously determined the relative activities of over 200 plant essential oils and their active components, phenolic compounds, and tea flavonoids against pathogenic bacteria (Friedman and others 2002, 2003, 2006a). The wide ranging bactericidal effects were quantitatively described in terms of BA_{50} values derived from concentration-activity plots and defined as the percentage of the sample that resulted in a 50% decrease in colony-forming units (CFU) under the test conditions. Selected compounds were also active against antibiotic-resistant bacteria and in commercial and freshly prepared apple juices (Friedman and others 2004a, 2004b, 2006b) as well as in ground beef (Juneja and others 2006).

The objectives of the present study were to evaluate bactericidal activities against *E. coli* O157:H7 and *Salmonella enterica* of selected active oils and oil compounds suspended in Chardonnay, Pinot Noir, and Sherry wines. Parameters expected to influence bactericidal activity, including the nature of antibacterial agent, the alcohol content, and acidity of the wines ; the effects of mixtures of antibacterial compounds, and the solubility of active compounds, added or extracted from oregano and thyme leaves, were examined. The results suggest that the described antimicrobial wine formulations merit evaluation for their ability to protect food against contamination by pathogenic organisms.

Materials and Methods

Materials

Oregano Spanish, oregano origanum, lemongrass, cinnamon cassia, and thyme plant essential oils were purchased from Lhasa Herb

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Company (Berkeley, Calif., U.S.A.). Dr. U. Ravid (Israel) provided the oregano Syrian oil. Compounds obtained from Sigma (St. Louis, Mo., U.S.A.) were carvacrol (m.w. 150), citral (m.w. 152; purity, 97%; 61% *cis* and 36% *trans* isomers), cinnamaldehyde (m.w. 134), and thymol (m.w. 150; purity, 99.5%). A second carvacrol sample was a gift of Millenium Specialty Chemicals (Jacksonville, Fla., U.S.A.). Dry oregano and thyme leaves (McCormick, Hunt Valley, Md., U.S.A.), Chardonnay 2002 (Wente, Livermore, Calif., U.S.A.), Pinot Noir 2003 (Beringer Founder's Estate, Napa, Calif., U.S.A.), and Dry Sherry (Christian Brothers, Cutler, Calif., U.S.A.) were purchased from local stores.

Solvents and buffers

The determined pH values and the percent ethanol on the labels for the 3 wines were Chardonnay, 3.3/13.5, Pinot Noir, 3.9/14.1, and Sherry, 3.3/18. Selected diluents used were saline pH 3.3; pH 3.8 (150 mM NaCl adjusted to acid pH with 1 N HCl); saline pH 3.8 + 14% of 95% ethanol; saline pH 3.3 + 19% of 95% ethanol, wine/saline (0.87 g NaCl added to 100 mL wine); and phosphate-buffered saline (PBS) pH 7.0 (prepared by mixing dibasic sodium phosphate [100 mM] and monobasic sodium phosphate [100 mM] at a 2:1 ratio, diluting by half with H_2O, and adding NaCl [150 mM]).

Sources of bacteria

The bacterial strains obtained from contaminated food and clinical sources used in this study were described previously: *E. coli* O157:H7 strain RM1484 and *S. enterica* serovar Hadar strain RM1309 (Friedman and others 2002).

Preparation of samples for bactericidal assays

Tubes (50 mL) containing 9.99 mL wine/saline and 10 μL oil (a 0.1% stock solution) for carvacrol, thymol, oregano, and thyme oils or 9.97 mL wine/saline and 30 μL oil for cinnamaldehyde, citral, cinnamon, and lemongrass oils (a 0.3% stock solution) were microwaved for 10 s and then shaken by hand 3 times. A sample (500 μL) of the solution or suspension was drawn immediately after shaking. An aliquot (500 μL) of the stock wine/saline/oil was added to 500-μL saline pH 3.3 (matched pH with Chardonnay and Sherry or pH 3.8, matched pH with Pinot Noir) for one-half dilution (Table 1). The dilution was then shaken before addition of another sample (500 μL) to the next tube in the series of 5 dilutions. In additional studies, the wine/saline/oil stocks were diluted with wine/saline pH 3.3/14% ethanol (95%) or saline pH 3.8/14% ethanol (95%).

Microtiter plates (96-well tissue culture plates from Nalge, Nunc, Rochester, N.Y., U.S.A.) were prepared prior to addition of bacterial suspensions. Saline acid pH 3.8 pH-matched control (100 μL) or test oil/wine saline other diluent were added to each of 6 wells. Aliquots (100 μL) of test wine/saline and their 5 dilutions were added to each of 6 wells. A total of 6 wells each were used for negative controls and for dilutions of test substances for sampling at 3 time intervals.

Bactericidal assays

The bactericidal assay described previously was adapted for this study (Friedman and others 2002). *E. coli* or *S. enterica* cells were stored on streaked Luria-Bertani agar (LB) plates (Difco, Sparks, Md., U.S.A.) and subcultured in LB broth for 16 to 18 h at 37 °C. A few isolated colonies were harvested from the plate with a sterile loop and suspended in 5 mL of LB in a 15 mL sterile plastic tube. The tubes were capped and incubated with shaking (200 rpm) at 37 °C for 18 h.

These bacterial suspensions were then prepared for the growth of CFU amounting to about 150 to 200 per lane on the square plates used for counting. A sample (1 mL) of an 18-h LB broth culture of the bacterium was added to a 1.9-mL microfuge tube and the bacteria were pelleted by centrifugation in a microfuge (15800 × *g*) for 1 min. The supernatant was removed and sterile PBS (1 mL, pH 7.0) was added to the pellet. The pellet was resuspended by gentle aspiration in and out of a transfer pipette. The sample's optical density at 620 nm was adjusted with PBS to approximately 0.4 (1/4 dilution, that is, 250-μL bacterial suspension plus 750-μL PBS). The suspension (20 μL) was added to PBS (980 μL). An aliquot of this suspension (80 μL of *E. coli* or 40 mL of *S. enterica*) was then added to 5 mL of saline pH 3.7 or other diluent. Saline pH 3.7 bacterial suspension (5 mL) or other diluent was vortexed and poured into a sterile plastic Petri Dish. Bacterial suspensions (50 μL) were drawn with a multichannel Eppendorf pipette (Hamburg, Germany) using 6 channels and added to 6 microtiter plate wells. This was repeated until all the prepared wells were inoculated.

Following incubation, samples (10 μL) from each of 6 wells were drawn with the pipette and 6 drops were spotted at the top of a square plate with grids containing LB agar for *E. coli* or *S. enterica*. A 10-μL spot per grid was chosen such that spots would not run together; that is, 6 spots were spaced across the top, 1 for each of the dilutions of wine/saline/oil test sample or undiluted negative controls. The plate was tilted and tapped gently to facilitate the movement of the liquid to the bottom of the plate. They were then covered and incubated overnight at 37 °C. CFU were enumerated after 18 to 24 h for each 10-μL streak using a colony counter. Experiments were initially done in duplicate using 2 pellets processed from 1 species. Selected experiments were repeated up to 10 times.

Bactericidal activities (BA_{50} values)

Bactericidal activities are defined as the percentage of test compound that caused a 50% reduction of CFU compared to a pH-matched control. Each compound was tested as a series of 6 dilutions, typically from 0.0021% to 0.067%. CFU values from each dilution were transferred to a Microsoft Excel 8.0 spreadsheet to determine the percentage of bacteria killed per well. Each of the dose-response profiles (percent test compound compared with percent bactericidal activity) was examined graphically and the BA_{50} values, corrected for purity, were estimated by a linear regression. The lower the BA_{50} and the higher the $1/BA_{50}$, the greater the activity.

Table 1 — Antimicrobial activity of wines containing 0.02% carvacrol in well against *E. coli* 0157:H7

	BA_{50}: μmoles/well[a]			
	Wine/saline pH 3.8	**Saline pH 3.8[b]/14% EtOH**	**Saline pH 3.8[b]**	**PBS pH 7**
Pinot Noir	0.12 ± 0.006[c]	0.45 ± 0.06[c]	0.82 ± 0.04[c]	0.94 ± 0.03[c]
Chardonnay	**Wine/saline pH 3.3**	**Saline pH 3.3[b]/14% EtOH**	**Saline pH 3.3[b]**	**PBS pH 7**
	0.11 ± 0.006	0.20 ± 0.02[c]	0.45 ± 0.02[c]	0.94 ± 0.03[c]

[a] BA_{50} — micromoles in microtiter well that caused a 50% reduction in CFUs. Listed values are averages from 4 separate determinations ± standard deviation ($n = 4$).
[b] Saline is pH matched to wine: pH 3.8 for Pinot Noir; pH 3.3 for Chardonnay.
[c] Statistical one-tailed tests were used to estimate an increase from the specified formulation with the lowest value. All BA_{50} values are significantly greater (the activity is lower) at the 5% level than the respective lowest values (0.12 for Pinot Noir and 0.11 for Chardonnay).

Solubility of oil compounds in wines and aqueous ethanol determined by HPLC

Suspensions of oil compounds were made by combining 1 mL each of cinnamaldehyde, citral, or carvacrol, or 1 g solid thymol into a 60-mL separatory funnel containing 25 mL of wine or aqueous ethanol. Samples were shaken vigorously for 1 min. The samples were left standing for 2 d. A total of 2-mL samples were taken from the middle of the vessel, and filtered through a 0.5-μm Millex-LCR13 (Millipore, Bedford, Mass., U.S.A.) syringe filter. The filtrate was diluted with 50% ethanol and then analyzed by an HPLC method adapted from a previously described procedure (Friedman and others 2004b). The HPLC system consisted of a Beckman 110B pump (Beckman Instruments, Berkeley, Calif., U.S.A.) and Thermo Separation Products AS3500 Autosampler (loop size, 100 μL) and UV 3000HR-scanning detector with both deuterium and tungsten lamps (Thermo Separation Products, San Jose, Calif., U.S.A.). The dimensions of the Supelco LC-ABZ column were 250 $\times$ 4.6 mm (plus a 2-cm precolumn). The particle size of the column packing was 5 μm. The degassed eluent consisted of 50% acetonitrile, 50 mM ammonium phosphate, and 0.05% phosphoric acid, pH 3.1. The flow rate of the pump was 1 mL/min. Injected sample volume was 20 μL. The absorbance was monitored at 3 wavelengths at or near the spectral maxima of the compounds of interest: citral, 240 nm; cinnamaldehyde, 280 nm; and carvacrol and thymol, 200 nm. All samples were analyzed in triplicate. A calibration curve was run daily using duplicate injections. The Thermo Separation Products PC1000 System Software (Thermo Separation Products) controlled the system and automatically calculated the solubility in mg/mL based on the calibration data.

Extraction of essential oil compounds from herbs into wines determined by HPLC

Dry oregano or thyme leaves (1 g) were weighed into a 60-mL separatory funnel. A total of 25 mL of wine or corresponding aqueous ethanol solution (determined from the label) was added to each leaf sample. Funnels were shaken for 10 s initially, then again in about 1 h. The samples were allowed to sit for 1 wk, then shaken again just before filtering through a 0.45-μm nylon membrane (Sigma-Aldrich, St. Louis, Mo., U.S.A.) with a 1.2-μm GF/C glass microfiber prefilter (Whatman, England). Carvacrol and thymol were quantitated by HPLC using authentic external standards (Sigma) as described above. Neat wine samples showed no interfering peaks. The experiment was run on 3 different days. Each listed value is an average $\pm$ SD for 3 replicates (3 separate experiments).

Statistical analysis. One-tailed Dennett's test was used to determine increases in BA_{50} values from the specified formulation with the lowest value. The Pinot Noir data were transformed by logs to stabilize the variance among formulations (SAS 2000).

Results and Discussion

Relative bactericidal activities of the wine formulations

Table 2 lists the experimental BA_{50} values for essential oil/oil compounds in wines at 3 time periods, 3, 15, and 30 min. To facilitate discussion of the trends in antimicrobial effectiveness, Figure 1 shows plots of $1/BA_{50}$ values (calculated from the BA_{50} listed in Table 2) for observed activities after an incubation time of 30 min. All compounds inhibited the growth of *E. coli* and *S. enterica*. The results will be described for each test sample.

Carvacrol and oregano oil. Carvacrol, the major component of oregano oil, is designated as Generally Regarded as Safe (GRAS) (Burt 2004). It is also used as a flavoring agent in baked goods, ice cream, beverages, and chewing gum (Fenaroli 1995).

We compared activities of carvacrol obtained from 2 sources: Sigma and Millenium. Table 2 and Figure 1 show that the BA_{50} values (percentage in microtiter well that induced a 50% reduction in CFU) of carvacrol obtained from Sigma in Chardonnay wine ranged as follows: $BA_{50} = 0.023$ after an incubation time of 3 min, 0.014 after 15 min, and 0.012 after 30 min. Carvacrol obtained from Millenium produced similar results. Comparison of antimicrobial effects of carvacrol in 2 other wines shows that Pinot Noir and Sherry wines induced similar bactericidal effects against *E. coli* as the Chardonnay solutions.

The BA_{50} values for *Salmonella* tended to be somewhat lower (activity was higher) than against *E. coli*. The high activities against both pathogens are striking. Thus, a BA_{50} value of 0.007 after incubation of 15 min means that a 0.007% solution of carvacrol in Sherry wine killed 50% of the *Salmonella*.

A total of 3 different oregano oils were evaluated: oregano origanum, oregano Spanish, and oregano Syrian. Table 2 shows that the BA_{50} values for oregano origanum oil in Chardonnay wine, activity against *E. coli* increased with time, with BA_{50} values of 0.033 after 3 min to 0.0099 after 30 min, about a 3-fold greater activity at the longer incubation time.

Similar trends are apparent for Pinot Noir and Sherry wines and for solutions of oregano origanum oils in 18% ethanol. The activities in the other 2 oregano oils are of the same order as those of oregano origanum oils. These observations suggest that the oil compounds act slowly during the 1st 2 to 3 min, but activity increases with time of incubation.

Both activities and trends for *S. enterica* seem to be similar to those described above for *E. coli*, with the possible exception of higher activities in Sherry compared to the other wines. Because oregano oil consists of approximately 80% carvacrol (Yannai 2004), it is not surprising that the observed trends in activities of the 3 oregano oils are somewhat lower than that of carvacrol. For example, for carvacrol in Pinot Noir wine, $BA_{50} = 0.008$ and for oregano origanum oil, 0.011, about a 30% difference. The observed antimicrobial activity of oregano oil can be largely accounted for by its content of carvacrol.

These results suggest that sources of the oregano oil do not seem to significantly affect activities against both pathogens and that commercially produced, less-expensive carvacrol could be used in food applications instead of oregano oils.

Cinnamaldehyde and cinnamon oil. Because cinnamaldehyde is present in commercial foods (Friedman and others 2000), has a pleasant taste, and is a GRAS flavoring agent (Adams and others 2004), the compound merits testing in antimicrobial formulations.

Table 2 and Figure 1 also show that the activity of cinnamaldehyde in the 3 wines against *E. coli* was much lower (the BA_{50} values are higher) than the corresponding activities of carvacrol. For example, the BA_{50} value of cinnamaldehyde in Chardonnay wine after 30 min incubation, 0.11, is about 10 times greater (the activity is lower) than the corresponding value for carvacrol, 0.012. The data in Table 2 also show an approximately similar 10-fold variation in activities for the other 2 wines.

The data show much greater activities of cinnamaldehyde solutions in the 3 wines at the 3 time periods for *S. enterica* than for *E. coli*. Thus, the activity of cinnamaldehyde in Chardonnay wine against *S. enterica* after 30 min ($BA_{50} = 0.033$) is 3.3 times greater than the corresponding activity against *E. coli* ($BA_{50} = 0.11$). The corresponding ratio of activities for the 2 pathogens exposed to cinnamaldehyde in Pinot Noir wine is 2.5, and for Sherry wine, 3.3. Activities after 30 min are similar to those observed after 15 min.

Table 2 also shows that the antimicrobial activity of cinnamon oil in Chardonnay wine against *E. coli* increased with incubation time at the 3 periods tested (3, 15, and 30 min) from a BA_{50} value of 0.15 to 0.066. Similar trends are apparent with the other 2 wines. Activities against *S. enterica* are about 1.5 greater than against *E. coli*. Activity of cinnamon oil against both pathogens is of the same order as that observed with cinnamaldehyde. These results are not surprising in view of the fact that cinnamon oil contains about 86% of cinnamaldehyde (Friedman and others 2004a; Friedman and others 2004b). The observed antimicrobial activity of cinnamon oil can be largely accounted for by its content of cinnamaldehyde.

Citral and lemongrass oil. Table 2 and Figure 1 show that wines containing citral (mixture of 2 isomers), prevalent in lemongrass oil, citrus fruits (Yannai 2004), ginger, and basil (Duke 1995), were active against both *E. coli* and *S. enterica*; the activity of citral was similar to corresponding activities of cinnamaldehyde in wine. The activity of citral was about 2 times greater than corresponding activity of lemongrass oil. The level of citral in lemongrass oil was previously determined in this laboratory to be approximately 86% (Friedman and others 2004b).

Thymol and thyme oil. Table 2 and Figure 1 show that thymol solutions in the 3 wines are highly active against both *E. coli* and *S. enterica*. The BA_{50} values of thymol in Chardonnay wine incubated for 3 min (0.028) are similar to those observed at 15 min (0.022) as well as 30 min (0.022). Unlike cinnamaldehyde, thymol appears to be a fast-acting antimicrobial.

Table 2 also shows that at 30 min, wine solutions of thymol are about 2 to 3 times more active against *S. enterica* than against *E. coli*. The activities of wine solutions of thyme oil are somewhat lower than the corresponding activities of thymol, the most prevalent ingredient of thyme oil. Activities of thymol and thyme oil are of the same order as those described above for carvacrol and oregano oil.

Effect of pH and ethanol content on antimicrobial activity of wines

Table 1 shows that the BA_{50} value (0.12 ± 0.006; $n = 4$) of a solution of 0.02% carvacrol in a 2:1 mixture of Pinot Noir wine/saline pH 3.8 buffer was about 4 times lower (the activity was higher) than the BA_{50} value (0.45 ± 0.06; $n = 4$) of carvacrol in saline pH 3.8 solution containing 14% ethanol (pH and the percentage of ethanol were matched to that of the wine). The data show that ethanol appears to contribute to the activity since the BA_{50} value of carvacrol in the saline without ethanol was 0.82 ± 0.04 ($n = 4$). Because the BA_{50} value in pH 7.0 PBS buffer was increased only to 0.94 ± 0.03 ($n = 4$), acid pH seems to make a minor contribution to the activity.

Comparison of parallel data shown in Table 1 obtained with Chardonnay wine indicates similar trends, except that decreases in activity were lower for the saline compared to the wine formulations.

Antimicrobial effects of mixtures

A need exists to ascertain whether mixtures of compounds will act additively or synergistically in killing bacteria, as described

Table 2—Bactericidal activities (BA_{50})[a] of essential oils/oil compounds against *E. coli* O157:H7 and *S. enterica* in Chardonnay, Pinot Noir, or Sherry wines incubated at 21 °C for 3, 15, and 30 min

		BA_{50} values[a] (percent/well)					
		E. coli			*S. enterica*		
Oil/oil compound	Wine	3 min	15 min	30 min	3 min	15 min	30 min
Carvacrol, Sigma	Chardonnay	0.023 ± 0.01^{b}	0.014 ± 0.004^{b}	0.012 ± 0.0001^{b}	0.029 ± 0.01^{b}	0.0098 ± 0.002^{b}	0.0081 ± 0.002^{b}
	Pinot Noir	0.03 ± 0.01^{b}	0.019 ± 0.01^{b}	0.016 ± 0.004^{b}	0.026 ± 0.01^{b}	0.011 ± 0.001^{b}	0.008 ± 0.001^{b}
	Sherry	0.02 ± 0.005^{b}	0.012 ± 0.002^{b}	0.011 ± 0.0007^{b}	0.021 ± 0.01^{g}	0.007 ± 0.002^{f}	0.0068 ± 0.003^{f}
Carvacrol, Millenium	Chardonnay	0.024 ± 0.002^{b}	0.2016 ± 0.005^{b}	0.015 ± 0.004^{b}	0.018 ± 0.007^{b}	0.011 ± 0.005^{b}	0.01 ± 0.005^{b}
	Pinot Noir	0.025 ± 0.01^{b}	0.021 ± 0.001^{b}	0.021 ± 0.002^{b}	0.019 ± 0.001^{b}	0.01 ± 0.002^{b}	0.0087 ± 0.002^{b}
	Sherry	0.022 ± 0.002^{b}	0.015 ± 0.004^{b}	0.012 ± 0.001^{b}	0.015 ± 0.005^{b}	0.0074 ± 0.002^{b}	0.0059 ± 0.0008^{b}
Oregano Oreganum Oil,	Chardonnay	0.033 ± 0.002	0.021 ± 0	0.022 ± 0.0007	0.024 ± 0.0007	0.012 ± 0.0007	0.0099 ± 0.0002
	Pinot Noir	0.033 ± 0.002	0.022 ± 0	0.023 ± 0.0007	0.024 ± 0.0007	0.012 ± 0.0007	0.011 ± 0
	Sherry	0.035 ± 0.006	0.023 ± 0.0007	0.023 ± 0.0007	0.023 ± 0.0007	0.012 ± 0.0007	0.011 ± 0
Oregano Spanish Oil	Chardonnay	0.036 ± 0.004^{b}	0.028 ± 0.01^{b}	0.022 ± 0.008^{b}	0.028 ± 0.009^{b}	0.016 ± 0.007^{b}	0.014 ± 0.005^{b}
	Pinot Noir	0.034 ± 0.009^{b}	0.028 ± 0.007^{b}	0.023 ± 0.007^{b}	0.027 ± 0.008^{b}	0.01 ± 0.0009^{b}	0.013 ± 0.006^{b}
	Sherry	0.038 ± 0.002^{b}	0.029 ± 0.01^{b}	0.029 ± 0.009^{b}	0.025 ± 0.01^{b}	0.008 ± 0.002^{b}	0.0079 ± 0.003^{b}
Oregano Syrian Oil	Chardonnay	0.04 ± 0.0007	0.034 ± 0.01	0.031 ± 0.01	0.04 ± 0.003	0.01 ± 0.0008	0.011 ± 0.0005
	Pinot Noir	0.037 ± 0.004	0.041 ± 0.002	0.031 ± 0.01	0.037 ± 0.004	0.0099 ± 0.0009	0.01 ± 0.001
	Sherry	0.039 ± 0	0.039 ± 0.001	0.031 ± 0.01	0.04 ± 0.003	0.011 ± 0.0004	0.0098 ± 0.002
Cinnamaldehyde	Chardonnay	$>0.2^{c}$	0.13 ± 0.01	0.11 ± 0.02	0.1 ± 0.01	0.036 ± 0.004	0.033 ± 0.0007
	Pinot Noir	>0.2	0.14 ± 0.007	0.13 ± 0.01	0.14 ± 0.001	0.063 ± 0.001	0.051 ± 0.01
	Sherry	0.14 ± 0.01	0.13 ± 0	0.098 ± 0.02	0.061 ± 0.03	0.038 ± 0.01	0.03 ± 0.01
Cinnamon Oil	Chardonnay	0.15^{d}	0.1 ± 0.04	0.066 ± 0.004	0.12 ± 0.007	0.048 ± 0.02	0.038 ± 0.0007
	Pinot Noir	0.17^{d}	0.11 ± 0.02	0.082 ± 0.01	0.14 ± 0.007	0.064 ± 0.01	0.05 ± 0.02
	Sherry	0.13 ± 0.007	0.073 ± 0.01	0.067 ± 0.003	0.093 ± 0.02	0.049 ± 0.02	0.057 ± 0.01
Citral	Chardonnay	>0.2	0.069 ± 0.008	0.065 ± 0.002	0.12 ± 0	0.034 ± 0.001	0.033 ± 0
	Pinot Noir	0.16 ± 0.04	0.077 ± 0.01	0.07 ± 0.006	0.075 ± 0.001	0.045 ± 0.01	0.038 ± 0.002
	Sherry	0.13 ± 0.03	0.066 ± 0.0007	0.06 ± 0.005	0.073 ± 0.02	0.042 ± 0.01	0.033 ± 0.003
Lemongrass Oil	Chardonnay	>0.2	0.067 ± 0.003	0.071 ± 0.009	0.13 ± 0.01	0.061 ± 0.004	0.053 ± 0.01
	Pinot Noir	0.15 ± 0.03	0.073 ± 0.006	0.067 ± 0.003	0.16 ± 0.05	0.069 ± 0.003	0.066 ± 0.004
	Sherry	0.12 ± 0	0.066 ± 0.003	0.059 ± 0.001	0.13 ± 0.002	0.065 ± 0.005	0.061 ± 0.006
Thymol	Chardonnay	0.028 ± 0.008	0.022 ± 0.0007	0.022 ± 0.0007	0.023 ± 0.01^{b}	0.0097 ± 0.001^{b}	0.0086 ± 0.002^{b}
	Pinot Noir	0.03 ± 0.008^{b}	0.018 ± 0.003^{b}	0.016 ± 0.005^{b}	0.018 ± 0.002^{b}	0.011 ± 0.0009^{b}	0.01 ± 0.0008^{b}
	Sherry	0.035 ± 0.007^{f}	0.025 ± 0.009^{f}	0.019 ± 0.007^{f}	0.026 ± 0.01^{f}	0.012 ± 0.009^{e}	0.0088 ± 0.002^{f}
Thyme Oil	Chardonnay	0.036 ± 0.003	0.033 ± 0.006	0.033 ± 0.001	0.039 ± 0.003	0.014 ± 0.005	0.011 ± 0
	Pinot Noir	0.035 ± 0.003	0.034 ± 0.001	0.033 ± 0.005	0.039 ± 0	0.011 ± 0.0009	0.0097 ± 0.002
	Sherry	0.041 ± 0.002	0.035 ± 0.004	0.039 ± 0.004	0.044 ± 0.002	0.013 ± 0.0009	0.0098 ± 0.0004

[a] BA_{50} — concentration of test substance that caused a 50% reduction in CFU. [b] $n = 2$, unless noted otherwise.
[c] > signifies no activity, that is, less than 50% reduction in CFU at the highest dose. [d] $n = 1$. [e] $n = 10$. [f] $n = 8$.

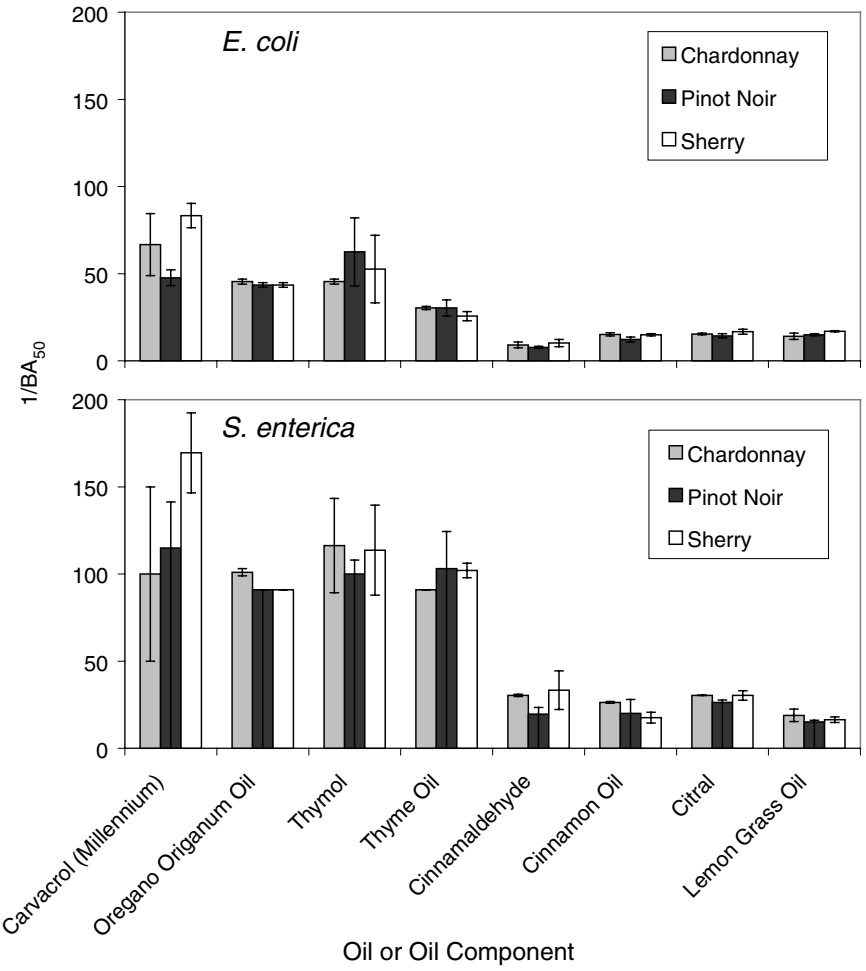

Figure 1 — Bactericidal activities (1/BA_{50} values) of essential oils and oil compounds in Chardonnay, Pinot Noir, and Sherry wine

elsewhere for biological effects of combinations of potato glycoalkaloids (Friedman and others 2005; Rayburn and others 1995). Thus, for mixtures of 2 compounds consisting of 33% A and 67% B, we would calculate amounts that kill 33% of the bacteria in combination with calculated concentration that would kill 67% of the bacteria. Synergism is thus defined as activities of the combinations, which are higher than the predicted additive activities of the individual compounds. Combinations that act synergistically will require smaller amounts of the ingredients to act as effective antimicrobial formulations.

In an exploratory study, we calculated the predicted activities for carvacrol/thymol in Sherry wine at 2:1 and 1:2 ratios. The experimental bactericidal values correspond to predicted values for additive effects against both *E. coli* and *S. enterica* (Table 3).

Wine extracts of oregano and thyme leaves

Figure 2 shows HPLC chromatograms of neat Pinot Noir wine and of ethanol and Pinot Noir wine extracts of oregano and thyme leaves. The chromatograms of the wine extracts contain peaks associated with carvacrol and thymol as well as additional peaks associated with unknown compounds. Analogous chromatograms were generated by Chardonnay and Sherry wine extracts (not shown). We do not know whether the unknown compounds would contribute to the antimicrobial activities of the extracts.

The data in Table 4 show that (1) the amounts of antimicrobials extracted from the oregano leaves with Sherry wine (104 μg/mL of carvacrol + 29.9 μg/mL of thymol; total = 134 μg/mL or 0.014%) approximate the 0.02% level of highly antibacterial carvacrol shown Table 1; (2) for Chardonnay wine, carvacrol = 76 μg/mL and thymol = 24.8 μg/mL, and for Pinot Noir wine carvacrol = 108 μg/mL and thymol = 32.7 μg/mL); and (3) the amounts extracted from thyme leaves were about two-thirds lower than the levels extracted from oregano leaves. Exploratory studies indicated that soaking oregano and thyme leaves overnight in wines produced wine formulations active against both *E. coli* O157:H7 and *S. enterica* (Table 4). Figure 3 shows that the amounts of carvacrol and thymol extracted from leaves after 1 wk exposure were only 10% to 15% higher than those obtained after 24 h extraction of the leaves shown in Table 4.

These exploratory studies suggest that oregano and thyme leaves widely used in culinary practices in homes and restaurants can

Table 3 — Additive effects carvacrol/thymol mixtures in Sherry wine on bactericidal activities (in μmoles/well) against *E. coli* O57:H7 and *S. enterica* at 21 °C after incubation for 30 min

Carvacrol + Thymol	*E. coli*	*S. enterica*
100% Carvacrol	0.70 ± 0.04*	0.68 ± 0.009
100% Thymol	0.98 ± 0.33	0.67 ± 0.05
67% Carvacrol/33% Thymol (actual)	0.99 ± 0.11	0.67 ± 0.05
67% Carvacrol/33% Thymol (predicted)	0.79	0.67
33% Carvacrol/67% Thymol (actual)	0.80 ± 0	0.69 ± 0.03
33% Carvacrol/67% Thymol (predicted)	0.88	0.67

*μmoles of test substance that induced a 50% reduction of bacteria in the microtiter well; $n = 2$.

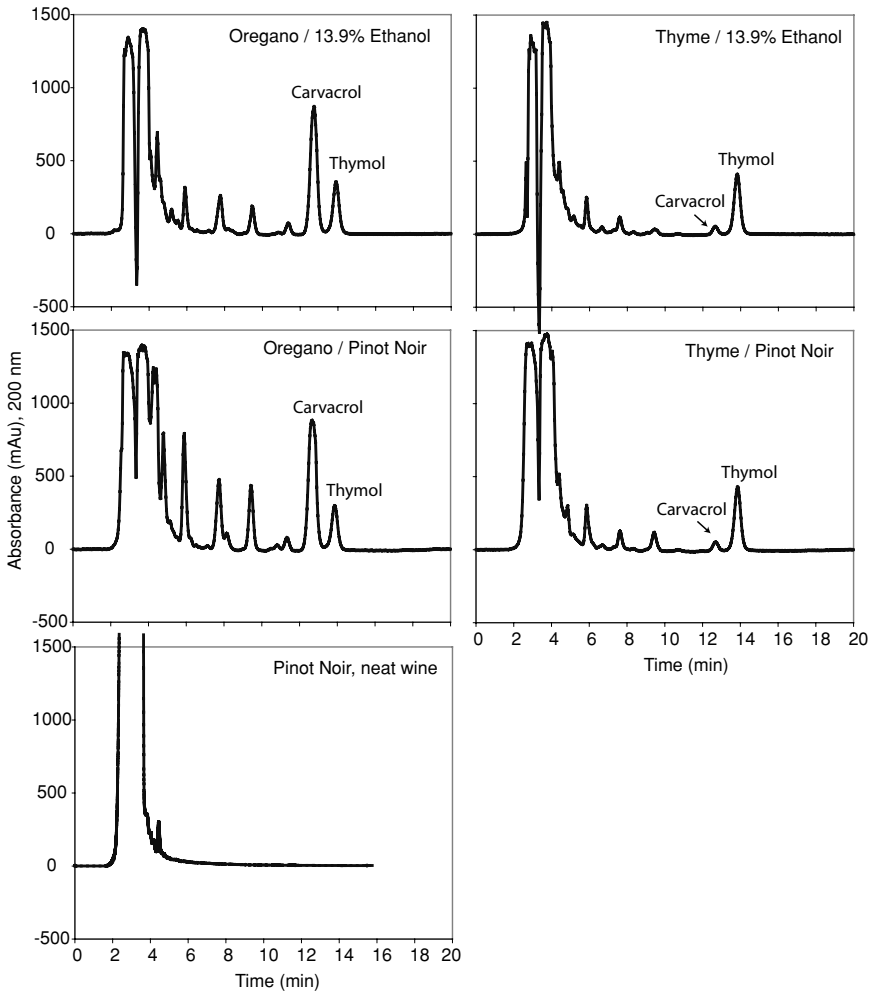

Figure 2 — HPLC chromatograms of oregano and thyme leaves extracted into Pinot Noir and matched ethanol solvents

provide a ready source of natural antimicrobials for the preparation of antimicrobial wine marinades. This aspect merits further study.

Solubility of oil compounds in wines

Table 5 shows the relationship of added carvacrol, cinnamaldehyde, citral, and thymol to the ultimate concentrations found in the final solutions of 3 wines and in aqueous ethanol, determined by HPLC. Solubility tended to increase with ethanol content and was generally somewhat greater in the wines than in the equivalent aqueous ethanol solvents, especially for cinnamaldehyde. The data also show that citral was the least and cinnamaldehyde the most soluble compound. In addition, there appears to be no apparent relationship between solubility and antimicrobial effects. Although carvacrol is less soluble in all 3 wines than is cinnamaldehyde, its antimicrobial activities are much higher (Table 2). It is likely that miscibility of the compounds in wine, rather than solubility, is more significant.

Conclusion

Wines appear to be useful solvents for plant-derived antimicrobial formulations. We found that several plant essential oils and oil compounds in Chardonnay, Pinot Noir, and Sherry wines are active against the foodborne pathogens *E. coli* and *S. enterica*. Exploratory studies with mixtures of 2 antimicrobials indicate that the effects are additive. We also found that wines can be used to extract the antimicrobials carvacrol and thymol from readily available oregano and thyme leaves. Because the formulations were tested under laboratory conditions, additional studies are needed to define their effectiveness, compatibility, sensory properties, and safety in various applications (Bena and Gasser 2004). The wine-oil antimicrobial solutions could be used as food washes or marinades to control foodborne bacterial pathogens. Because the components are GRAS, as opposed to harsh chemicals such as bleach or detergents, these formulations are preferable.

Table 4 — Bactericidal activities (BA_{50} values)[a] of wine/24 h leaf extracts against *E. coli* O157:H7 and *S. enterica* in Chardonnay, Pinot Noir, or Sherry incubated at 21 °C for 30 min, and composition of those extracts determined by HPLC

				Active leaf component extracted into wine (μg/mL)	
Extract		***E. coli***	***S. enterica***	**Carvacrol**	**Thymol**
Oregano leaves	Chardonnay	45 + 2.1[b]	30 + 8.9[c]	76.0	24.8
	Pinot Noir	>67[d]	35 + 5.8[c]	108	32.7
	Sherry	46 + 2.8	33 + 5.9[c]	104	29.9
Thyme leaves	Chardonnay	>67	40 + 4.2	4.7	34.1
	Pinot Noir	>67	57 + 1.4	4.1	31.4
	Sherry	>67	46 + 1.4	4.9	38.6

[a] BA_{50} — percent of wine extract that caused 50% reduction of CFU.
[b] $n = 2$ unless noted otherwise.
[c] $n = 4$.
[d] 67% of wine extract did not kill 50% of the bacteria.

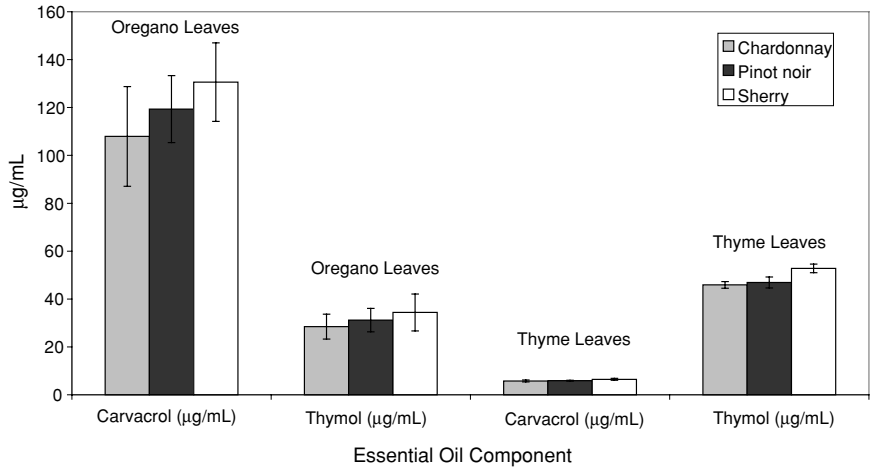

Figure 3 — Carvacrol and thymol extracted from leaves into wines over a 7-d period

Table 5 — Solubilities of essential oil compounds in aqueous-ethanol and in wines with equivalent ethanol content. Listed values (in mg/mL) are averages ± SD ($n = 3$).

Oil compound	13.5% EtOH	Chardonnay wine, 13.5% EtOH	14.1% EtOH	Pinot Noir wine, 14.1% EtOH	18% EtOH	Sherry wine, 18% EtOH
Carvacrol	0.69 ± 0.011	0.86 ± 0.031	1.05 ± 0.017	1.04 ± 0.009	1.22 ± 0.011	1.24 ± 0.017
Cinnamaldehyde	1.01 ± 0.010	1.11 ± 0.002	1.16 ± 0.016	1.41 ± 0.010	1.68 ± 0.027	2.13 ± 0.003
Citral	0.04 ± 0.000	0.02 ± 0.000	0.51 ± 0.022	0.61 ± 0.001	0.69 ± 0.012	0.72 ± 0.002
Thymol	0.72 ± 0.025	0.78 ± 0.005	1.08 ± 0.010	1.18 ± 0.038	1.36 ± 0.045	1.45 ± 0.016

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